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HUMIC ACID INVESTIGATIONS

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The alkali-soluble, acid-insoluble portion of soil organic matter is a complex group of polyanions containing bound protein fragments. The composition of these fragments is fairly well known,[1] but the way they are associated with the non-protein portion still remains obscure, as does the nature of the polyanions themselves.[2]

Three processes are thought to make the major contribution to the formation of the complex. They are:

(*a*) browning reactions both in the presence and absence of nitrogenous compounds;
(b) tanning of proteins by plant tannins;
(c) complexing of proteins and other nitrogenous compounds with lignin products.[3]

Some efforts have been made in recent years to fractionate the polyanion complex by classical[2] and paper electrophoresis,[4,5] and paper chromatography.[5]

Long chain quaternary ammonium compounds (detergents) have been used to precipitate polysaccharide polyanions.[6] These detergents solubilize proteins and have been used to dissociate proteins from their acid polysaccharide complexes.[7]

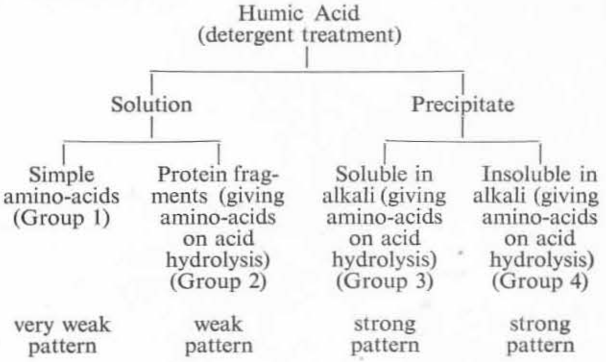
We found cetyltrimethyl ammonium bromide precipitates a considerable proportion of humic acid from aqueous solution. Cetyl pyridinium chloride produces hydrophobic precipitates soluble only in organic solvents. Humic acid was prepared by the classical method (Achard), extracting air-dried peat with 0·5N sodium hydroxide and precipitating with 1N hydrochloric acid (cf. Ref. 8). Several re-precipitations followed. This older method was used in preference to pyrophosphate extraction to avoid possible pyrophosphate interference, although it was realized that some degradation might result.[9]

Cetyltrimethyl ammonium bromide solution (10%) in water was added slowly to a slightly alkaline (NaOH) solution of the humic acid preparation (0·4%) until further addition of the cationic detergent gave no precipitate. After leaving overnight the precipitate was separated (centrifuge). The precipitate was washed free of excess detergent. The bulk of the detergent in the supernatant liquid was removed by potassium iodide precipitation followed by shaking with chloroform.[7]

Two dimensional paper chromatography[10] showed that a small quantity of a few free amino-acids (aspartic acid, serine, glycine, alanine and valine) were solubilized by the detergent and somewhat larger quantities were found in the hydrolyzate (6N hydrochloric acid, 24 hours, 100°, sealed tube) of this fraction (aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, lysine, histidine, arginine, valine+methionine, methionine sulphoxide, methionine sulphone, phenylalanine, leucines and two unknowns).

The precipitate did not dissolve in strong potassium chloride solution, contrary to the behaviour of the detergent-acidic polysaccharide complex noted elsewhere[7] and was found to be only partially soluble in 0·5N sodium hydroxide (Soluble portion A: Insoluble portion B). Hydrolyzates (6N hydrochloric acid, 24 hours, 100°, sealed tube) of the two fractions (A and B) showed strong amino-acid patterns similar to that of the original humic acid (A: aspartic acid, glutamic acid, 3 : 4 dihydroxyphenylalanine (d.o.p.a.), serine, glycine, threonine, alanine, tyrosine, valine + methionine, phenylalanine, leucines, arginine, lysine, histidine, proline and an amino-sugar. B: no d.o.p.a. or histidine spots).

Table I


Humic Acid
(detergent treatment)
Solution
Precipitate
Simple amino-acids (Group 1)
Protein fragments (giving amino-acids on acid hydrolysis) (Group 2)
Soluble in alkali (giving amino-acids on acid hydrolysis) (Group 3)
Insoluble in alkali (giving amino-acids on acid hydrolysis) (Group 4)
very weak pattern
weak pattern
strong pattern
strong pattern

The humic acid fraction of soil organic matter is precipitated by long chain quaternary nitrogen detergents, as are other polyanions (Table I). The complex with these detergents is stable towards electrolytes unlike the usual acid polysaccharide complex. This may point either to a different type of linkage between detergent and polyanion, or merely to a lower solubility of the complex than is the case with acidic polysaccharides.

The relative strengths of the amino-acid patterns suggest that only a small quantity of free amino-acids and a minor portion of the protein fragments are loosely held (Groups 1 and 2, Table I); the major portion of the protein fragments is distributed between two fractions in which it is strongly bound (Groups 3 and 4, Table I). This is consistent with the incorporation of protein fragments into complexes formed by the three major processes.

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